Soil Treatments for the Control of the Japanese Beetle Larvae

By L. B. Smith,
In Charge Japanese Beetle Project.

When the nursery quarantine was first applied several years ago it was realized that an embargo on the movement of nursery stock with soil about the roots, out of the Japanese beetle area would work a serious hardship on nurserymen and others located in the area infested or which would soon be infested. The policy was adopted that providing treatments were found which would entirely free the soil from infestation, plants with soil about the roots would be certified for shipment after they had been treated and all infestation destroyed. It then became a problem of devising methods of soil treatment which would give a complete kill of the grubs without injury to plants. At the time these investigations were undertaken little was known concerning treatment of soil under such conditions, and much preliminary work had to be done before a method was found which would serve the purpose desired. After various measures had been tried without success the possibilities narrowed down to the use of a chemical. Long and detailed experiments were performed with many materials. This resulted in developing the use of carbon bisulphide (CS_2) as a soil insecticide for the control of the Japanese beetle. It was found that exceedingly small dosages of this chemical were extremely toxic to the immature stages of the insect and to obtain a uniform distribution through the soil it became necessary to employ some dispersing agent, such as water. Since carbon bisulphide is relatively insoluble in water a homogenous emulsion was prepared which contains 71.4 per cent. carbon bisulphide. The dilution and volume of dilute solution applied to the soil depend upon the soil temperature, kind of soil, class of plant to be treated, etc. An

emulsion of oil of wormseed was also found to possess high toxicity on the Japanese beetle grubs. It is more costly than carbon bisulphide and is used only in the case of a few species of matted-root perennials which will not withstand treatements of carbon bisulphide.

The methods followed in treating plants with soil about the roots vary somewhat according to the variety of plant. In principle they are much the same. It is unfortunate that the major papers dealing with these methods of soil treatment are in press and have not as yet been published.

Experimental Data.

A brief summary of the results secured during the past five years from the investigations on soil insecticides at the Japanese beetle laboratory follows:

The purpose of this presentation is to indicate to those unfamiliar with the work the quantity of data on which the conclusions are based, that these treatments sufficiently safeguard the movement of nursery stock with soil. One of the difficulties encountered in the early investigations was the fact that only a limited number of nurseries were infested, and it was a problem many times to obtain sufficient naturally infested material for experimental work. This resulted in methods being devised for infesting artificially soil balls and potted plants in such a way as to closely approximate natural conditions. In using an insecticide on soil about the roots of plants the concentration is limited by two conditions: 1st, the maximum dosage is limited to the amount which can be used without injury to the plant; 2nd, the minimum dosage is limited by the amount and concentration necessary to kill any larvae present under

the conditions of treatment. In all the treatments which have been applied we have maintained the policy of using as large a dosage as the plants would stand, often times going so far as to cause a certain amount of injury to the plants. This was done to allow a wide margin of safety between the actual dosage and the amount necessary to kill the grubs.

As an example of the methods used in insecticide experiments, let us assume it was desired to find a method for treating ferns, such as Cybotiums. These plants are grown in specially prepared soil, which renders treatment more difficult. A series of plants which may be growing in 4 inch pots or 10 or 12 inch tubs are artificially infested with the grubs. Larvae are placed in the tubs by punching small holes with a dibble into the pot ball and placing the grubs therein. To insure that the grubs have become established in the pot ball 2 to 6 weeks are allowed after they are placed before the treatments are applied. In a series of experiments using 10 to 12 inch tubs, between 10 and 25 grubs are placed in each tub. The treatment consists of pouring a volume of liquid into the pot equivalent to the volume of the ball and allowing it to percolate through the soil. The application is made in one of two ways: 1st, by using a galvanized iron collar 8 or 9 inches tall, which is placed around the top of the pot and the entire amount of liquid poured in at once; 2d, the solution may be poured through the soil ball using 2 or 3 successive applications. Another series of plants which have not been infested with the grubs are treated in a similar way. Still another series, including both uninfested and infested plants, are treated with water. After treatment the plants are undisturbed for 48 hours, at which time the soil balls are

broken up and the number of grubs killed is determined. Careful check is kept on the condition of the uninfested plants for some time after treatment, since even small amounts of the carbon bisulphide may cause a temporary set-back to certain types of plants through the destruction of many of the finer roots. In general it has been much easier to destroy the larvae with carbon bisulphide emulsion than to safely treat the plants.

Minimum Lethal Dosage.

In finding the minimum lethal dosages which will destroy all larvae in the soil, over 500 series of experiments were performed and not less than 10 grubs were used in any single test. With the temperature at 70° F. a dosage of 0.375 cc. of carbon bisulphide to a liter of water was necessary to obtain a complete kill of the eggs, larvae and pupae of the Japanese beetle. This is equivalent to 30 cc. of carbon bisulphide emulsion containing 71.4% of carbon bisulphide diluted in 16 gallons of water. The following tabulated statement summarizes the dosages which have been found to give a complete kill at various temperatures:

Soil temperature	60° F. or above	- 2-1/2cc.	of carbon bisulphide emulsion to 1 gallon of water
" "	55-60° F.	- 3.125 cc.	" " " " " "
" "	50-55° F.	- 3.75 cc.	" " " " " "
" "	42-50° F.	- 4.375 cc.	" " " " " "
" "	35-42° F.	- 5.0 cc.	" " " " " "

These represent the dosages used in commercial treatments, and are from 20 to 50 per cent. higher than is necessary to obtain a complete kill of the grubs in sandy loam soil. Special potting soils containing from 30 to 75 per cent. peat require higher dosages. It will be observed that an ample margin of safety is maintained in the dosages used under commercial conditions. In

addition to the experiments to determine the lethal dosages on the insects, it has been necessary to treat experimentally thousands of out-door-grown plants in order to find a maximum dosage which could be safely applied. The dosages in all cases are just below the concentration causing injury to the plants. In fact, certain varieties, such as hydrangeas, suffer a slight injury from the treatment. Such injury is not permanent and the roots usually grown out again in 2 to 3 weeks.

Many varieties of potted plants require a volume of dilute carbon bisulphide emulsion equivalent to one-half of the volume of soil. Under certain special conditions, such as in the case of Cybotiums and other plants grown in soil containing a large proportion of peat, it has been necessary to use a volume of liquid equivalent to the total amount of soil.

Treatment of Evergreen stock.

Effective results have been obtained in the experiments on the treatment of evergreen trees in the row by carbon bisulphide emulsion. An attempt was first made, after the plant had been dug, to submerge the soil ball in the dilute carbon bisulphide emulsion. This was not successful since the liquid penetrates into the ball of soil, largely displacing the air from the pore space on the outside of the mass. When the pore space is filled the movement of the liquid into the soil practically ceases, much of the CS_2 is absorbed by the soil and it has been impossible under experimental conditions to obtain complete diffusion of the insecticide throughout the soil ball by the dipping method. In view of the limits of the dipping method it became a problem of devising means to obtain adequate diffusion of the insecticide through the soil ball.

Other experiments were conducted in the nurseries, which developed what is called the "pouring method." A galvanized iron collar, 9 inches wide and of sufficient length to enclose the area about the tree from 12 to 18 inches larger than the soil ball, is sunk in the ground to a depth of 3 inches, and the soil banked upon the outside. The insecticide is poured into the container thus formed, and allowed to percolate through the soil about the roots on the plant. One of the most difficult problems was engendered by the fact that very few nurseries, growing evergreen trees, have had suffieient infestation of larvae for experimental work. This necessitated artificially infesting the plants. As a result a great many of the experiments have been performed on trees infested in this manner. Since this method of treatment contemplates an undisturbed condition of the soil, holes could not be punched through the surface soil in order to place the grubs in the vicinity of the tree roots without danger of disturbing the top soil, and thereby render it more easy for the liquid to penetrate. To offset this danger trenches were dug at a distance from the tree and small holes punched through the soil from the side of the trench at various depths. Grubs were carefully placed at the end of the tunnels, which came under the area to be treated. The holes were then plugged and the trenches refilled. This avoided any disturbance of the surface soil in the area to be treated. The maximum depth at which the grubs were placed was 18 inches. From a series of experiments including the treatment of 150 infested trees in the field, it was found that 1.8 gallons of liquid to each square foot of soil gave 100% kill of larvae 18 inches below the surface. As an instance of

the effectiveness of the carbon-bisulphide emulsions, it was found that toads 20 inches deep in the soil near the trees were killed by a solution containing 0.5 cc. of carbon bisulphide to each liter of water. In determining the penetration of the emulsion in various types of soil, 26 series of experiments were run, including over 500 evergreen trees growing in soil types from heavy clay to light sandy loam. These were performed under actual nursery conditions. It was found that in no case did the kill amount to less than 100% at depths of 18 inches below the surface. The average winter depth of the larvae is 4 to 6 inches, and in the spring and autumn when the treatments are applied they are usually from 1/2 to 1-1/2 inches deep. Records are rare of any larvae having been found at a depth of more than 12 inches.

Comparison of treatments of field grown and potted stock.

The treatment of plants growing in the field has always been more satisfactory than the treatment of potted stock. Under experimental conditions the pot treatment has been found satisfactory but when such treatments were applied under commercial conditions many difficulties arose. It is difficult to determine the condition within the pot ball of plants repotted several times in various types of soil. This may render it difficult to obtain a uniform penetration of the liquid through the ball. It is also felt that the abnormal physical features of the root-bound pot ball may influence the drainage, and there is danger not only of injuring the plant but of some larvae not being killed under commercial treatments. Since these difficultues do not apply in the case of field grown plants, it is felt that the treatment of plants in the field is as nearly fool-proof as any chemical treatment could possibly be.

Treatment of Matted-root Perennials.

Japanese iris, peonies and perennial phlox are all extensivley grown in the area now infested with the Japanese beetle. While these plants are not shipped with soil about the roots, the nature of their root structure is such as to make it almost impossible to inspect every plant unless they are thoroughly washed, an operation which injures the roots appreciably. In the case of peonies the roots are filled with many cavities formed underground by the flower stems of the previous year's growth. These become filled with soil and larvae are frequently found in them. As a result, a study was undertaken some time ago to discover a solution in which these plant roots could be dipped, after the greater amount of soil had been removed, which would kill any larvae present, without injury to the plants. The result was the development of an emulsion of oil of wormseed, the active principle of which is ascaridole. In treating peonies, iris, phlox and sedum plants, it is found advisable to pack the plants in tubs until they are nearly full, then completely covering them with the emulsion. Galvanized iron tubs are used for this purpose since they rarely leak and do not absorb any of the toxic material from the dip. In cold weather the plants are held in a room at a temperature of 70° F. for 24 hours previous to treatment and all treatments are made at this temperature or above.

Japanese iris, phlox, and sedum are treated with the wormseed oil emulsion, and the dosage is based on the ascaridole content of the wormseed oil. The dosage consists of 1 cc. of ascaridole to 6 liters of water and the plants are submerged in the emulsion for 15 hours. Peony roots are treated with carbon bisulphide emulsion, using a dosage of 0.5 cc. of carbon bisulphide to each liter of water, and the period of treatment is 15 hours

At the end of the period of submergence, the plants are removed from the dip, the latter discarded and the plants, after draining, maintained in a room at 70° F. for 48 hours. In determining the toxicity of wormseed oil to the Japanese beetle larvae, large series of experiments were performed, using an oil containing 75% ascaridole as determined by the acetic acid method. Since it became necessary to disperse the wormseed oil in water, various hydrophile colloids were tested as possible emulsifiers. The emulsion finally used was one consisting of 10 parts by volume of caustic potash fish oil soap, 20 parts of wormseed oil, and 10 parts of water. The soap is dissolved in hot water and mixture added to the wormseed oil. Temperature is an extremely important factor in treatments with wormseed oil emulsion, and it was found throughout the series of experiments that exposures of 4 hours in length at 70° F. gave 100% kill. In order to maintain an ample margin of safety in all of the commercial treatments, such treatments are required to be applied for not less than 15 hours.

Methods of commercial treatment.

Under Appendix A of the Quarantine Regulations now in effect on account of the Japanese beetle, under the title of "Certification of Requirements for each Class of Nurseries", it states, "The only distinction in the restrictions as to shipments from nurseries of Class 2 and 3, either to points entirely outside of the regulated area or to nurseries of Class 1, is the provision for shipments from nurseries of Class 2 of plants with soil under the conditions specified in HB-178. No shipments with soil are permitted by nurseries of the third class except as indicated in HB-178 in the cases of certain classes of plants where it is possible to treat the soil under methods which have been

determined as being 100 per cent. effective in killing the grubs."

In view of the fact that it was considered a sufficient safeguard to chemically treat the soil about the roots of plants for the destruction of the larvae of the Japanese beetle it became necessary to develop an organization competent to perform and supervise the treatments in connection with the quarantine division. It has been the policy of the Laboratory that the matter of treatments could not and should not be turned over to the nurserymen. As a result a man was selected from the research division who was experienced in the investigational phases of soil insecticides and who was thoroughly familiar with all phases of the soil treatments. He was placed in charge of the commercial treatment of plants in the various nurseries, under the immediate supervision of the quarantine officer in charge. This expert on soil treatment in turn has skilled inspectors under his direction to apply the treatments under the conditions specified. In no case is the responsibility for the treatment left to the discretion of the nurserymen. The nurserymen are required in most cases to furnish the materials necessary for making the treatments and a sufficient amount of labor to assist in the general performance of the work. Not only is the actual operation of treating the plants controlled by the Laboratory, but to a large extent the cultural treatment of the plants previous to treatment which might in any way influence the results obtained is rigidly controlled through the quarantine service. Should any condition arise during the course of treatment which might lead to any suspicion that any treatment might not be 100% successful, it leads to immediate refusal of certification for those particular plants. As an instance, one nurseryman requested the treatment of about two thousand

azalea plants. Owing to extremely dry conditions it was impossible to obtain a thorough penetration of the liquid to all portions of the soil ball. Later examination revealed this condition and the result was a refusal to certify these plants for shipment. In all cases a certain proportion of the plants are carefully examined after treatment to observe whether any condition has existed which might form a cause for doubt as to the efficiency of the treatment.

In the treatment of matted-root perennials the following general requirements are made: (1) The temperature of the carbon bisulphide emulsion during the entire period of treatment must be maintained at 70° F. or higher. During the seasons when the plant roots are cold, they must be kept in a temperature of at least 70° F. for a period of at least 24 hours previous to treatment, and after treatment they must be maintained at 70° F. for 48 hours; (2) Plants should not be dug for treatment immediately after a rain, nor should they be placed in water previous to treatment; (3) Large clumps of roots must be broken up into sizes permitting ready penetration of the emulsion; (4) The carbon bisulphide emulsion must be used but once, it is then discarded and the tank thoroughly cleaned before using again; (5) Containers must be kept tightly covered during the entire period of treatment. These regulations apply also to the treatment of plants with wormseed oil emulsion.

Ornamental grasses are treated with 37 cc. of prepared carbon bisulphide emulsion to each 14 gallons of water. The time of treatment is 15 hours and the temperature must be maintained at 70° F. Rhubarb roots are treated for the same length of time at the same temperature, using 50 cc. of carbon

bisulphide emulsion to 16 gallons of water. Blueberries grown in the open are treated with 2½ cc. of carbon bisulphide emulsion to 1 gallon of water and the applications are made at the rate of 1.8 gallons to each square foot of soil.

In the treatment of evergreens, such as Arbor Vitae, Spruce, Fir, Hemlock, etc., 2½ cc. of emulsion are used to 1 gallon of water when the soil temperature if 60° F. or above. As the soil temperature drops below 60° F., the dosage is increased according to the table previously mentioned. In all cases at least 1.8 gallons of solution are used to treat one square foot of soil. Trees recently transplanted will not be treated. All plants must be thoroughly established in the soil before treatments are made. In making the treatments the lower branches of the trees are lifted and tied close to the trunk. The soil around the tree to be treated must be level when the cylinder is placed. If the soil in the crown about the roots is higher than the surrounding soil, it must be removed. The galvanized iron cylinders are sunk into the ground to a depth of 3 inches and the soil banked up on the outside to prevent leakage of the solution from underneath. After the solution has completely percolated into the soil, the collars are removed and the soil which was banked against the cylinder is pushed away. The treatment is complete in 48 hours after application and the trees must be dug at that time; otherwise, certification will not be made.

The fumigation of potting soil with carbon bisulphide for the control of the Japanese beetle.

The experimental work leading to the adoption of carbon bisulphide

as a fumigant of potting soil was conducted at the Japanese Beetle Laboratory and the results of these investigations are published in Bulletin 380 of the New Jersey Agricultural Experiment Station, January, 1923. The general summary of the results is, that carbon bisulphide is a cheap and efficient material for fumigating potting soil to free it from infestation by the Japanese beetle larvae. The minimum lethal dosage in liter of air or relatively dry soil when the temperature is above 50° F., and the exposure is 24 to 48 hours is 0.04 g. Soil air moisture does not effect the minimum lethal dosage to any appreciable extent. The efficiency of the minimum lethal dosage varies directly with the temperature, being non-effective below 50° F. The efficiency of the minimum lethal dosage varies inversely with the soil moisture, being non-effective in wet soil. Carbon bisulphide must be placed in the soil to kill all the larvae. It is advisable to use not less than 13 fluid ounces (1 pound) to a cubic yard of soil, allowing an exposure of 48 hours at temperatures of 50° F. or above. The dosage holes must be arranged so that all of the diffusion regions from the various injection holes overlap, and thus assure all parts of the soil being treated. When 18 inches of soil have been placed in the fumigation box, injection holes should be made 6 inches deep, 12 to 15 inches apart and 12 to 15 inches distant in the rows, with 1-1/2 ounces of carbon bisulphide in each injection hole. The hole is closed after making the application, and 18 inches more soil is placed in the box and the process repeated until the box is filled. The concentration of the gas varies directly with the depth from the surface of the soil and inversely with the distance laterally from the injection hole. Maximum concentration is obtained sooner and disappears more quickly from the layers of soil relatively near the surface. Lateral diffusion of the gas varies directly with the atmospheric temperature, but is independent

generally speaking of the atmospheric pressure. The soil takes up or absorbs a considerable amount of the initial carbon bisulphide charge, after which it removes little from the soil air. Retreatment greatly increases the persistence of the vapors in the soil. The amount of carbon bisulphide recommended for soil fumigation has little or no effect on subsequent plant growth.

Nurserymen desiring to fumigate potting soil are required to build special tight fumigation chambers. These are usually concrete and are fitted with tight covers. Careful supervision is maintained by the Laboratory on all treatments of such soil.

Fumigation of the soil ball with CS_2 vapor.

This discussion thus far has been concerned largely with the method of treatment with carbon bisulphide dispersed in water. During the past year a method of exposing the soil balls or pot balls, as the case may be, to the vapor of carbon bisulphide has proven an exceedingly efficient method of soil treatment. This is done in a special chamber in which the plants are inverted over a tank of water. The tops of the plants are submerged and thereby protected from the vapors of the gas, and the soil balls are subjected to the treatment for periods of 8 to 12 hours. While this method is still in the experimental stage, it is one of the most promising ideas yet developed for the control of the grubs in the roots of nursery stock. The advantage of this method is that the conditions governing the effectiveness of the treatment can be controlled to a large extent. Before it is used commercially, however, further work is necessary to insure its effectiveness under all the varied conditions which can arise. Its use will be largely in the treatment of certain potted plants which it is impossible to treat with the dilute emulsion. It may also be used in the treatment of certain types of evergreen

stock up to 6 or 7 feet in height.

Do chemical treatments sufficiently safeguard shipments of plants with soil about the roots?

In closing it may be well to summarize the conditions on which the argument is based that adequate restrictions are enforced to prevent any infestation being sent out from the regulated area.

Large amounts of potted nursery stock are grown under conditions where no possible infestation could take place. This includes ferns, palms, roses, and many similar plants. Those of you who are familiar with the regulations are aware that the nurserymen are required to screen their houses thoroughly during the season when the adult beetle is in flight. This means that all ventilators, doors, windows, or other openings in the house must be screened and protected in such a way that there is no possible chance of any adult beetle gaining access to the house. A group of inspectors is maintained, during the season of active flight of the beetle, whose duty it is to inspect greenhouses several times a week to make sure that these regulations are being followed and that these protections are being maintained. All soil which is used in screened houses is chemically treated or sterilized with steam to destroy all living larvae of the Japanese beetle. The houses protected in this manner are free from infestation and the stock sent out from them is safe, as far as the Japanese beetle is concerned, as though it was grown outside of the infested area.

Another class of stock includes all plants with soil about the roots which are grown in districts which have not as yet been infested with the Japanese beetle. Careful supervision and inspection of all such nurseries is maintained and the surrounding territory is thoroughly scouted during the

summer to determine the presence of the beetles if any should occur. Careful and systematic diggings are made in the soil of any nurseries in or near which any adult beetles are found. Therefore, stock from such nurseries can not be considered otherwise than as safe and free from any infestation.

The third class of stock consists of potted plants grown in situations whereby they are likely to become infested with the grubs of the Japanese beetle. On the basis of recent experience and despite the fact that satisfactory results were obtainedunder experimental conditions, it is felt that there is a remote danger in the commerical application of pot treatments. We have every reason to believe such treatments would render the plants, 99.9% free from infestation; nevertheless we are refusing certification until improved methods of treatment have been worked out which will give absolute assurance that such stock is free from any infestation whatsoever.

The fourth class of stock, includes hydrangeas, and other plants which are grown in the field, potted in the autumn and shipped in pots during the winter. These can be treated in the field before they are potted, by a method similar to the one used in the treatment of conifer stock.

As has been explained, all treatments are made under the direct supervision of trained men and an effective organization. There is no question of a nurseryman in the rush of business slighting matters pertaining to the regulations in the least. Whether the nurseryman ships his stock out on time is not our concern until he has complied with the regulations. Our duty is to see and insure that the stock is free from infestation before certifying it for shipment.

The fifth class includes large amounts of evergreen stock, such as Arbor Vitae, Pines, Hemlocks, etc. These plants can be treated standing in the row by means which we firmly believe <u>give greater protection</u> than would

be given could the plants be freed from soil and the roots inspected. There are few quarantines, operated to prevent the spread of an insect or a plant disease, where certification has depended on the visual inspection of the plants, which has ever been 100% effective. In the case of the Japanese beetle quarantine on nursery stock which has been in operation for five years, and plants of various kinds have been treated and shipped for the past three years, there has not been one instance known of a single grub being carried out on this stock. This record would seem to justify the policies which have been maintained and points to the efficiency of the organization operating the quarantine on account of this insect.